at blocking transcription of proneural genes when transcription becomes autoregulatory, interrupting fate determination at this crucial juncture (Baker et al., 1996; Culi and Modolell, 1998). This is a further example where the protein-protein interactions between transcription factors determine the biology. As zur Lage et al. point out, it may prove important whether any Ato/HES interactions exclude interactions such as that between Ato and Pointed. Evidently, further insights into the mechanisms of cell fate determination lie ahead.

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Selected Reading

Baker, N.E., Yu, S., and Han, D. (1996). Curr. Biol. *6*, 1290–1301.

Culi, J., and Modolell, J. (1998). Genes Dev. *12*, 2036–2047.

Giagtzoglou, N., Alifragis, P., Koumbanakis, K.A., and Delidakis, C. (2003). Development *130*, 259–270.

Sieweke, M.H., Tekotte, H., Jarosch, U., and Graf, T. (1998). EMBO J. *17*, 1728–1739.

Slack, J.M.W. (1983). From Egg to Embryo (Cambridge, UK: Cambridge University Press).

Sun, Y., Jan, L.Y., and Jan, Y.N. (1998). Development *125*, 3731–3740.

zur Lage, P.I., Powell, L.M., Prentice, D.R.A., McLaughlin, P., and Jarman, A.P. (2004). Dev. Cell *7*, this issue, 687–696.

Bub1, a Gatekeeper for Cdc20-Dependent Mitotic Exit

The mitotic spindle assembly checkpoint arrests cells at metaphase by suppressing Cdc20, a protein required to trigger ubiquitination and consequent degradation of cyclin B. New evidence from Tang et al. appearing in the November 5th issue of *Molecular Cell* finds that one of the checkpoint proteins, Bub1, specifically phosphorylates Cdc20 to suppress APC/C activation.

The cell cycle is subject to a number of checkpoint controls that function to preserve the genome by restraining progression until prerequisite events have been properly completed. From yeast to mammals there are spindle assembly checkpoints that read proper alignment and tension of chromosomes in the mitotic spindle before anaphase can initiate. The system appears to function by the recruitment of a group of checkpoint control proteins, including (in higher eukaryotes) Mad1, Mad2, Bub1, Bub3, Mps1, and BubR1, to the kinetochores, and ablation or suppression of function of any of these proteins substantially compromises mitotic checkpoint control (Lew and Burke, 2003). Ultimately, the checkpoint operates by sequestering Cdc20, a key regulator of the anaphase promoting complex/cyclosome (APC/C), a complex that functions to ubiquitinate two key substrates, securin and cyclin B, tagging them for proteosome destruction, that in turn is the critical event permitting mitotic exit (Peters, 2002).

Three of the checkpoint control proteins, Bub1, Mps1, and BubR1, are protein kinases. It has been reasonable to assume that kinase activity is intimately connected to checkpoint function, but the crucial substrates these checkpoint proteins regulate, that make sense with respect to checkpoint control, have been lacking. Indeed, the kinase domain of BubR1 appears to be dispensable for its APC/C inhibitory activity (Tang et al., 2001). Given the number of checkpoint control proteins involved, it would appear that the control network, once unraveled, would turn out to be highly complex. Cdc20 phosphorylation has recently received attention for its potential in regulating APC/C activation (Chung and Chen, 2003), but the protein kinase identified, MAPK, is not one of the checkpoint kinases.

Contrary to the expected complexity of checkpoint control, a striking and important paper by Tang et al., appearing in the November 5th issue of *Molecular Cell*, has shed unexpected light on the mechanism by making a clear linkage between the kinase activity of one of the checkpoint control proteins, Bub1, and Cdc20 control of APC/C activation. In this paper, the authors have established that, for mammalian cells, there are six phosphorylation sites on Cdc20 that are phosphorylated by Bub1, but not by BubR1, MAPK, or a battery of other kinases. Further, mutation of these phosphorylation sites to alanine creates a dominant-negative effect, with reduced checkpoint arrest in mitosis. Going in the other direction, the authors have established that Bub1 ablation, or expression of a Bub1 dead kinase, abolishes Cdc20 phosphorylation and also suppresses the spindle assembly checkpoint.

As the authors point out, the spindle assembly checkpoint is exquisitely sensitive, responding to a single off-plate chromosome or to loss of tension in properly aligned chromosomes. The existence of such catalytic checkpoint machinery, as described here, offers a highly sensitive response mechanism that should permit the necessary rapid amplification of signal.

While this work establishes a clear pathway by which the spindle assembly checkpoint may at least partly control APC/C function, it opens many important questions. Issues of great interest include how this pathway fits with the essential functions of the other checkpoint control proteins and of Cdc20 phosphorylation by other protein kinases (Chung and Chen, 2003). Further, it will be important to address what controls Bub1 so that it maintains Cdc20 in phosphorylated status only during checkpoint arrest. Bub1 and Cdc20 participate in a multiprotein complex composed of other checkpoint

control proteins (Sudakin et al., 2001), and the explicit control described here will need to be put in context of regulation of the entire complex, since all the proteins play essential roles in checkpoint control.

In addition, some mitotic checkpoint controls are apparently independent of kinetochore recruitment of the checkpoint proteins and appear to depend on Mad2 rather than Bub1 (Martin-Lluesma et al., 2002; Skoufias et al., 2004). What happens to Cdc20 phosphorylation status under these circumstances? Are there additional protein kinase controls or phosphatase-dependent controls that come into play?

A particularly appealing aspect of this paper is that it shows a strong interplay between regulatory phosphorylation and protein degradation. Two fundamental regulatory mechanisms of cell cycle progression are protein phosphorylation, particularly by the cyclin-dependent kinases (Forsburg and Nurse, 1991), and the specific proteosome-dependent destruction of regulatory proteins such as cyclins (Hershko, 1997). It would stand to reason that there must be nodal points in cell cycle control where these two major mechanisms intersect in the performance of key regulatory controls. This paper stands as one clear example of such a regulatory interaction.

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Selected Reading

Chung, E., and Chen, R.H. (2003). Nat. Cell Biol. *5*, 748–753.

Forsburg, S.L., and Nurse, P. (1991). Annu. Rev. Cell Biol. *7*, 227–256.

Hershko, A. (1997). Curr. Opin. Cell Biol. *9*, 788–799.

Lew, D.J., and Burke, D.J. (2003). Annu. Rev. Genet. *37*, 251–282.

Martin-Lluesma, S., Stucke, V.M., and Nigg, E.A. (2002). Science *297*, 2267–2270.

Peters, J.M. (2002). Mol. Cell *9*, 931–943.

Skoufias, D.A., Lacroix, F.B., Andreassen, P.R., Wilson, L., and Margolis, R.L. (2004). Mol. Cell *16*, 977–990.

Sudakin, V., Chan, G.K., and Yen, T.J. (2001). J. Cell Biol. *154*, 925–936.

Tang, Z., Bharadwaj, R., Li, B., and Yu, H. (2001). Dev. Cell *1*, 227–237.

Tang, Z., Shu, H., Oncel, D., Chen, S., and Yu, H. (2004). Mol. Cell *16*, in press, 387–397.